CLINICAL STUDY

Expression pattern of the pendrin and sodium/iodide symporter genes in human thyroid carcinoma cell lines and human thyroid tumors

Franco Arturi, Diego Russo¹, Jean-Michel Bidart², Daniela Scarpelli, Martin Schlumberger² and Sebastiano Filetti

Dipartimento di Medicina Sperimentale e Clinica, ¹Dipartimento di Scienze Farmacobiologiche, Università di Catanzaro, 88100 Catanzaro, Italy and

²Institut Gustave Roussy, 94805 Villejuif, France

(Correspondence should be addressed to S Filetti, Cattedra di Endocrinologia, Dipartimento di Medicina Sperimentale e Clinica, Via T. Campanella, 115-88100 Catanzaro, Italy; Email: filetti@tin.it)

Abstract

Objective: In the present study we analyzed the pattern of pendrin (PDS) and sodium/iodide symporter (NIS) gene expression in some thyroid carcinoma cell lines and a series of thyroid tumoral tissues.

Methods: Total RNA was extracted from all cell lines and from 53 tissues, and gene expression was examined by RT-PCR. Semiquantitative 'multiplex' RT-PCR was used to assess variations in PDS gene expression among various thyroid pathologies. Pendrin expression was determined in the thyroid cell lines by Western blot analysis.

Results: PDS mRNA was expressed in all the cells investigated; conversely, NIS mRNA was detectable only in the B-CPAP cells. Pendrin protein was expressed in B-CPAP and WRO cell lines, reduced in FRO and absent in ARO cells. PDS gene expression was not detected in 5 of 25 differentiated thyroid carcinomas (DTC) while NIS gene was not expressed in six carcinomas. A concordance expression of both PDS and NIS transcripts was found in 20 DTC. In contrast, 2 neoplastic thyroid tissues carrying undetectable PDS mRNA maintained NIS transcript, and 3 thyroid carcinomas negative for NIS mRNA retained the expression of PDS gene. A semiquantitative analysis showed that the mean PDS mRNA levels were significantly decreased in DTC tissues.

Conclusions: Our data demonstrate that pendrin expression: (i) is present in the more differentiated thyroid carcinoma cell lines studied; (ii) is reduced or absent in DTC tissues; (iii) may not correlate with the NIS expression. These alterations may contribute to the loss of iodine concentration ability detected in thyroid tumors.

European Journal of Endocrinology 145 129–135

Introduction

Highly specialized proteins regulate iodine metabolism and hormonogenesis in thyroid cells. A number of these proteins have already been identified and functionally characterized. This complex machinery appears imbalanced during thyroid neoplastic transformation. Indeed, a variety of iodine metabolism abnormalities have been demonstrated in thyroid cancer tissue (1). Moreover, a loss of iodide trapping ability appears to be an early feature during oncogenic transformation of thyroid cells *in vitro* (2). Recently pendrin, a highly hydrophobic transmembrane protein composed of 780 amino acids, has been recognized as a chloride and iodide transporter (3), suggesting a potential role in thyroid iodine metabolism. Indeed, the PDS gene, encoding pendrin, is highly expressed in thyroid tissue (4, 5), although its role in thyroid pathology is not fully clarified. However, pendrin is detectable at the apical

membrane of the thyrocytes; this localization, as well as its functional activity *in vitro*, supports its role in iodide transport into the follicular lumen (5, 6). Mutations of the PDS gene are responsible for Pendred syndrome, an autosomal recessive disease characterized by goiter and congenital deafness (7). Furthermore, in a previous study we found a variability in PDS gene expression in various thyroid disorders (5). However, whether pendrin plays a role in the iodine derangement occurring in thyroid carcinoma has yet to be clarified. On the other hand, several studies have already demonstrated a loss or decrease of the sodium/iodide symporter (NIS) gene expression in both primary and metastatic differentiated thyroid carcinomas (8–10).

The relative contribution of defective NIS and/or PDS expression in determining the reduced or absent iodine-concentrating ability in thyroid cancer cells remains to be established. In this regard, we analyzed the expression of the PDS gene and pendrin in a number of

human thyroid carcinoma cell lines carrying various degrees of malignancy. In addition, we evaluated PDS mRNA levels and compared PDS and NIS gene expression in a series of thyroid tumors, including benign and malignant primary and metastatic carcinomas.

Materials and methods

Cell culture and iodide uptake

The human thyroid cell lines used in this study were B-CPAP (11) (from papillary carcinoma, provided by A Fusco, University of Catanzaro, Italy), WRO (12) (from follicular carcinoma, provided by G Juillard, UCLA, USA), FRO (13) and ARO (12) (both from anaplastic carcinoma, provided by G Juillard). All cell lines except the ARO were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin/streptomycin and amphotericin B. The ARO cell line was grown in RPMI 1640. As positive control, we used normal thyroid cells in continuous culture.

Iodide uptake assay was performed as described previously by Weiss *et al.* (14). Each experiment was done at least twice in quadruplicate and the FRTL-5 cells were used as positive controls.

Human thyroid tissues

Forty-one thyroid tumors and twelve normal thyroid tissues collected at the Gustave Roussy Institute (Villejuif, France) and at the University of Catanzaro (Italy) were examined. Tissues obtained at surgery were immediately frozen and stored in liquid nitrogen until analysis. Tumors were histologically classified according to the World Health Organization (WHO) recommendations (15). The local ethical committee approved this work.

RNA extraction and RT-PCR

Total RNA was extracted from cell lines using the Qiagen RNA/DNA kit (Qiagen S.p.A, Milano, Italy), following the manufacturer's instructions. Total RNA was extracted from frozen fresh tissues using the RNA Btm technique (Bioprobe Systems Richmond, CA, USA), following the manufacturer's instructions as described previously (16). The tumor tissues used for RNA isolation in this study were microdissected by the pathologist to exclude contamination of surrounding normal thyroid cells.

Complementary DNA (cDNA) was synthesized from 1 µg total RNA according to the manufacturer's instructions (Roche Diagnostics SpA, Monza, Italy), as described previously (17). The mixture was incubated at 25 °C for 10 min, at 42 °C for 60 min, heated to 99 °C for 5 min, and then stored at -20 °C. PCR amplification was performed using 5 µl cDNA, as

described previously (18). The samples were subjected to 40 cycles of amplification and PCR conditions for the PDS gene were as follows: denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min and extension at 72 °C for 1 min. Ten of 50 µl of the amplification products were then run on 1.5% Tris-borate-EDTA agarose gel containing ethidium bromide and were analyzed to confirm a positive or negative outcome.

Primer oligonucleotides for the PDS gene (PDS2S-2A) were: 5' primer, 5'-CGATGGGAACCAGGAATTCA-3' and 3' primer, 5'-TCTCAGGACCACAGTCAACA-3'. The amplification yielded a 400 base pair DNA product corresponding to fragment 1361-1760, according to the sequence of the PDS cDNA reported in the GeneBank (4). The PDS primers spanned exon-intron junctions of the gene to exclude genomic DNA contamination. For the thyroglobulin (Tg), thyroperoxidase (TPO) and NIS gene amplification, we used the primer oligonucleotides previously described (17, 18). All primers were from Life Technologies S.r.l., San Giuliano Milanese (MI), Italy.

Multiplex semiquantitative RT-PCR

In human thyroid tumors, a semiquantitative 'multiplex' RT-PCR in the same tube was designed to compare the RT-PCR products of the PDS gene with β -actin gene products to determine the relative expression levels of these genes.

The PCR amplification was performed using 5 µl cDNA as described previously and the samples were subjected to 32 cycles of amplification. The PCR conditions were as follows: denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min and extension at 72 °C for 1 min.

Primer oligonucleotides for the PDS gene (PDS1S-1A) were: 5' primer, 5'-AGCAGAGACAGGTCATGGCA-3' and 3' primer, 5'-ATCCGACAGGAAGTGCAGCT-3'. The amplification yielded a 357 base pair DNA product corresponding to fragment 211-567 according to the sequence of the PDS cDNA reported in the GeneBank (4). Primer oligonucleotides for the β -actin gene were: 5' primer, 5'-CGAGGCCAGAGCAAGACA-3' and 3' primer, 5'-CACAGCTTCTCCTTAATGTCACG-3'. The amplification yielded a 482 base pair DNA product corresponding to fragment 209-691 according to the published sequence of the β -actin gene (19).

The PDS and β -actin primers spanned exon-intron junctions of the gene to exclude genomic DNA contamination. The reaction conditions were optimized to ensure that the amplification for both PDS and β -actin remained within the exponential range, by assessing the variation in signal intensity for PDS and β -actin at various cycle numbers. Three different ratios of primers were tested to get the same efficiency of amplification, and the ratio 1:1 was used (data not shown).

Ten of 50 µl of the amplification products were run on 1.5% Tris-borate-EDTA agarose gel containing

ethidium bromide. The bands of the positive film were scanned and the density and the width of each PCR product was measured using the NIH Image Program (Wayne Rasband, National Institutes of Health, USA).

Western blot analysis

Total proteins were extracted from thyroid cell lines as follows. Confluent cells from 3 Petri dishes were collected and homogenized in 1 ml buffer containing 250 mmol/l sucrose, 10 mmol/l HEPES-KOH (pH 7.5), 1 mmol/l EDTA, 1 mmol/l phenylmethylsulfonylfluoride, 10 µg/ml leupeptin, 10 µg/ml aprotinin. The homogenate was centrifuged at 14 000 *g* (4 °C for 15 min) and the supernatant (which contained the whole cell lysate) was quantified spectrophotometrically using the Bradford method (20).

Thirty micrograms proteins were loaded on a 4–20% gradient SDS-polyacrylamide gel and subjected to electrophoresis at a constant voltage (110 V). Electrophoresis to a Hybond ECL-PVDF nitrocellulose membrane (Amersham S.r.l., Milano, Italy) was performed for 2 h at 125 mA using a Mini Trans blot electroblotting system (Bio-rad Laboratories S.r.l., Milano, Italy). Blocking was done using TTBS/milk (1% Tween 20, TBS (20 mmol/l Tris-HCl, 150 mmol/l NaCl; pH 7.5), and 5% non fat dry milk) for 2 h at room temperature. The membrane was then incubated with a 1/1500 dilution of affinity-purified rabbit anti-pendrin polyclonal antibody (5) or a 1/5000 dilution of mouse monoclonal anti-human β-actin antibody, overnight at 4 °C in TTBS/milk. After one 15-min and two 5-min washes in TTBS, the membrane was incubated with a 1/2000 dilution of a horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody in TTBS/milk. After one 15-min and two 5-min washes in TTBS, the protein was visualized by an enhanced chemiluminescence Western blot detection system (ECLplus, Amersham S.r.l.).

Statistical methods

The expression of PDS transcripts detected in the different histotypes was compared with that in normal thyroid tissues. The significance of differences between group means was analyzed by Student's (unpaired) *t*-test. A level of $P < 0.05$ was considered statistically significant.

Results

Iodide transporters expression in human thyroid carcinoma cell lines

None of the thyroid carcinoma cell lines used in this study, comprising a spectrum ranging from well differentiated to undifferentiated phenotypes (11–13),

Table 1 I^- uptake in human thyroid carcinoma cell lines. The results are the means $\pm$ s.e. of two determinations.

Cell type	I^- uptake (pmol/µg DNA)
B-CPAP	1.68 $\pm$ 0.1
WRO	0.75 $\pm$ 0.2
FRO	0.82 $\pm$ 0.1
ARO	0.49 $\pm$ 0.2
FRTL-5	33.2 $\pm$ 0.9

I^- uptake was determined as described in Materials and methods.

Figure 1 Presence of NIS and PDS in human thyroid carcinoma cell lines. Agarose gel electrophoresis of RT-PCR-amplified products. Lane 1, normal thyroid cell line (positive control); lane 2, CHO cell line (negative control); lane 3, B-CPAP cells; lane 4, ARO cells; lane 5, FRO cells; lane 6, WRO cells. The expected bands are indicated by black arrows. The specific band for NIS gene was visualized using 5 additional cycles of PCR.

Figure 2 Western blot of human pendrin and β-actin using protein extracts from human thyroid carcinoma cell lines. Lane 1, positive control (proteins from Graves' tissue); lane 2, WRO cells; lane 3, FRO cells; lane 4, ARO cells; lane 5, B-CPAP cells. Thirty micrograms protein were loaded on a 4–20% gradient SDS-polyacrylamide gel under reduced conditions. A representative of two separate experiments is shown.

was able to concentrate iodine (Table 1). To examine whether the expression of the two molecules known to act *in vitro* as iodide transporters are altered in these cell lines, an RT-PCR analysis was performed. Figure 1 shows detectable levels of PDS transcript in all cell lines; conversely, the expression of the NIS mRNA was detected only in the B-CPAP cell line. To assess whether the PDS transcript detected in all cells was truly translated into the native protein, a Western blot analysis using a specific antibody directed against pendrin was performed. As shown in Fig. 2, a band of approximately 95 kDa corresponding to pendrin

Table 2 PDS and NIS expression in human thyroid tumors.

Patient number	Sex/age	PDS	NIS	Met/RIU	Tg (off T ₄)	Patient number	Sex/age	PDS	NIS
Papillary carcinomas						Toxic adenomas			
1	F/46	+	+	neg		26	F/49	+	+
2	M/36	+	+	neg		27	M/51	+	+
3	F/63	+	+	neg		28	M/59	+	+
4	F/37	+	—	neg		29	M/59	+	+
5	F/46	+	+	neg		30	F/45	+	+
6	M/75	—	—	+/-	Pos	Benign cold adenomas			
7	M/34	—	+	neg		31	F/29	—	+
8	M/55	+	+	neg		32	M/41	+	+
9	F/23	+	+	+/+	Pos	33	F/29	+	—
10	F/52	+	+	neg		34	M/63	+	+
11	M/22	—	—	+/-	Pos	35	F/43	+	+
12	F/56	+	+	neg		36	M/20	+	+
13	F/33	+	+	neg		37	M/40	+	+
14	F/25	—	—	+/-	Pos	38	F/49	+	+
15	F/41	+	—	neg		39	F/39	+	+
16	F/28	+	+	+/-	Pos	40	F/27	+	+
17	F/40	—	+	+/-	Pos	41	F/15	+	+
18	F/28	+	+	+/-	Pos	Normal thyroid tissues			
19	F/35	+	+	+/+	Pos	1	F/62	+	+
Follicular carcinomas						2	M/71	+	+
20	F/53	+	—	+/-	Pos	3	F/71	+	+
21	M/57	+	+	+/-	Pos	4	F/56	+	+
22	F/65	+	+	neg		5	F/36	+	+
23	F/55	+	+	+/+	Pos	6	F/46	+	+
24	M/28	+	+	+/+	Pos	7	M/42	+	+
25	F/28	+	+	+/+	Pos	8	M/53	+	+
						9	F/40	+	+
						10	F/28	+	+
						11	M/36	+	+
						12	F/58	+	+

Met, metastatic tissue; RIU, ¹³¹I uptake in metastatic tissue; neg, absence of metastases; Pos, Tg value >1 ng/ml.

protein was detected in the B-CPAP and WRO cell lines, was just visible in FRO cells and was absent in the ARO cell line.

Pendrin mRNA expression in human thyroid tumor tissues

The pattern of expression of both PDS and NIS genes was also explored in a series of normal and tumoral thyroid samples. All tissues investigated displayed the Tg and TPO transcripts (data not shown), indicating therefore the integrity of the mRNA and the cDNA obtained from tissue specimens.

PDS mRNA was detected in all normal thyroid tissues as well as in toxic adenomas and follicular thyroid carcinomas, even though it was at different expression levels. On the other hand, PDS gene expression was not detected in 5 of 19 papillary carcinomas (nos 6, 7, 11, 14 and 17) and in 1 of 11 hypofunctioning thyroid adenomas (no. 31) (Table 2).

Three of five human thyroid carcinomas negative for the PDS gene expression were also negative for NIS transcripts (nos 6, 11 and 14). Conversely, 2 carcinomas negative for PDS mRNA maintained the expression

of NIS gene (nos 7 and 17). Moreover, 3 carcinomas negative for NIS mRNA maintained the expression of PDS gene (nos 4, 15 and 20) (Table 2). Expression of PDS gene was also investigated in the metastatic tissues from two patients (nos 17 and 18) (Table 2). In both cases we found the same behavior as in the corresponding primary carcinomas (negative for PDS and positive for NIS in no. 17; positive for both PDS and NIS in no. 18) (data not shown).

A semiquantitative RT-PCR assay was used to analyze PDS mRNA levels. Mean levels were expressed

Figure 3 Semiquantitative 'multiplex' RT-PCR. A semiquantitative RT-PCR has been used to compare the RT-PCR products of the PDS gene with β -actin gene products to determine the relative expression levels of the PDS transcript (see Materials and methods). Some of the human thyroid tissues examined are shown: 1, normal thyroid tissue; 2 and 3, toxic adenomas; 4 and 5, papillary carcinomas; 6, follicular carcinoma; 7, cold adenoma.

Figure 4 PDS mRNA levels in different human thyroid tissues. The bars represent the mean $\pm$ S.D. PDS mRNA levels in the thyroid tissues examined (the same as in Table 1 except the negative samples). Levels of PDS gene are expressed relative to β -actin mRNA as described in Materials and methods. Bar 1 ($n = 12$), normal thyroid tissues; bar 2 ($n = 14$), papillary thyroid carcinomas; bar 3 ($n = 6$), follicular thyroid carcinomas; bar 4 ($n = 10$), cold thyroid adenomas; bar 5 ($n = 5$), toxic adenomas.

relative to the β -actin mRNA, used as internal control (Fig. 3). The PDS mRNA levels were variable among normal thyroid tissues as well as in tumoral thyroid tissues. The mean PDS mRNA levels were significantly lower in papillary and follicular thyroid carcinomas than in normal thyroid tissues ($P < 0.001$) (Fig. 4). Among carcinomas, mean levels of expression were significantly lower in follicular carcinomas than in papillary carcinomas ($P < 0.005$) (Fig. 4). Also, PDS gene expression was lower in hypofunctioning thyroid adenomas than in normal thyroid tissues ($P < 0.001$) (Fig. 4). A slight, but not significant decrease in PDS gene expression was observed in toxic adenomas (Fig. 4).

In the light of a possible clinical significance of our findings, we examined primary thyroid cancer specimens obtained from a group of Tg-positive and Tg-negative post-therapeutic radioiodine whole body scan (WBS) patients (nos 6, 11, 14, 16, 17, 18, 20 and 21, Table 2). The majority (5 out of 8) of the samples did not express one or both of the two genes studied. More interestingly, in one case (no. 17, Table 2) the metastatic tissue negative at the post therapeutic WBS was also investigated, and neither the metastasis nor the primary tumors expressed the PDS gene, while the NIS transcript was fully detected. Furthermore, PDS gene expression, when present, was markedly reduced (data not shown).

Discussion

The molecular mechanisms underlying the reduction/loss of ability to concentrate iodide in human thyroid tumors are not completely understood. Two proteins capable of transporting iodide *in vitro*, NIS and pendrin, have recently been identified and their genes have been

cloned. These proteins are expressed in the thyroid gland as well as in several other tissues (4, 9). However, it is not completely clear how pendrin displays its iodide transporter function in thyroid cells. In both benign and malignant human thyroid tumors, several studies have demonstrated a decrease or a loss of the NIS mRNA and protein expression (17, 21–25) in the absence of abnormalities of the structural gene (26). Nevertheless, some thyroid tumors maintain NIS expression despite the loss of iodide concentrating ability, suggesting that additional alterations may be involved. However, the relative contribution which alterations to NIS and/or pendrin make to the defective iodine metabolism of neoplastic thyroid tissue is unclear.

In the present study, first, we examined the expression of NIS and pendrin in some human thyroid carcinoma cell lines carrying a varying degree of malignancy (11–13). We previously demonstrated that oncogene-transformed thyroid cell lines show a reduction or absence of NIS gene expression (2). Similarly, NIS mRNA was not detectable in any of the human cell lines examined except the B-CPAP cell line in which its expression was very low. Accordingly, no iodide uptake was found in these human cell lines. In contrast, messenger RNA for PDS was expressed in all the cell lines investigated. Moreover, Western blot analysis showed different levels of expression of pendrin in the various cell lines, with the more differentiated ones (B-CPAP and WRO) presenting the higher amounts, and the less differentiated cells showing remarkably reduced (FRO) or absent (ARO) pendrin expression levels. These data, therefore, suggest that different alterations, also related to the more or less aggressive phenotype, may affect PDS and NIS gene expression and suggest a key role *in vitro* of NIS rather than of pendrin in the iodide uptake by neoplastic thyroid cells.

Next, by examining primary thyroid carcinomas, PDS gene expression was not detected in 5 of 19 papillary carcinomas. However, the absence of PDS transcript was not restricted to the malignant phenotype, being also found in one hypofunctioning benign follicular adenoma. Furthermore, a semiquantitative analysis of the PDS gene expression showed that the mean PDS mRNA levels were significantly decreased in human thyroid carcinomas and the mean levels of the PDS transcript were significantly lower in follicular carcinomas than in papillary thyroid carcinomas. Unfortunately, the absence of sufficient amount of tissue meant that the study of the protein expression in this series of thyroid tumors was not possible.

In addition, when we compared PDS and NIS transcripts in the 25 differentiated thyroid carcinomas, a concordant expression of both genes was found in 17 samples. This is in agreement with our previous data in which a positive relationship was found between PDS and NIS gene expression in another series of differentiated thyroid carcinomas (5, 23). Indeed, lack of expression of both PDS and NIS genes was found in

three thyroid carcinoma samples. In contrast, it is noteworthy that two papillary thyroid tumors showing undetectable PDS mRNA levels, maintained NIS gene expression. Conversely, three thyroid tumors negative for NIS mRNA transcript retained the expression of PDS gene.

Although the molecular mechanisms of these alterations are not known, our data show a differential expression pattern of the PDS and NIS genes in human thyroid cancers. On the other hand, the two gene products, at least in thyroid cells, display a distinct localization, NIS and pendrin being localized in the basolateral membrane and in the apical border of thyrocyte respectively. This probably reflects their distinct function, NIS being primarily involved in thyroidal iodide uptake from the blood, whereas pendrin mainly acts to deliver intracellular iodide into the follicular lumen.

Furthermore, PDS gene expression was reduced in this group of thyroid carcinomas. The relevance of this observation has yet to be established, but it suggests the possibility that, even though the iodide symporter is active, the impairment of the organification system (pendrin or even TPO molecules) may result also in a decreased ability to concentrate iodide. Indeed, alterations of the pendrin expression by compromising the efficiency of the iodide organification process may decrease the intrathyroidal iodide retention. This could affect, therefore, the efficacy of radioiodine treatment of thyroid carcinoma metastases. In conclusion, a variety of abnormalities in iodine metabolism, including alterations in pendrin expression, may contribute to the loss or reduction of the radioiodine concentrating ability by thyroid cancer tissue.

Acknowledgements

We acknowledge the support of Associazione Italiana per la Ricerca sul Cancro (AIRC) (to S Filetti), MURST PRI '99 (to S Filetti), MURST '00 (to D Russo), and Electricité de France (M Schlumberger). F Arturi is recipient of a 'Dottorato di ricerca in Basi Molecolari dell'azione ormonale' fellowship at University of Catania.

References

- 1 Thomas-Morvan C, Carayon P, Schlumberger M, Vignal A & Tubiana M. Thyrotrophin stimulation of adenylate cyclase and iodine uptake in human differentiated thyroid cancer. *Acta Endocrinologica* 1982 **101** 25–31.
- 2 Trapasso F, Iuliano R, Chiefari E, Arturi F, Stella A, Filetti S *et al.* Iodide symporter gene expression in normal and transformed rat thyroid cells. *European Journal of Endocrinology* 1999 **140** 447–451.
- 3 Scott DA, Wang R, Kremar TM, Sheffield VC & Karniski LP. The Pendred syndrome gene encodes a chloride–iodide transport protein. *Nature Genetics* 1999 **21** 440–443.
- 4 Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M *et al.* Pendred syndrome is caused by mutations in a putative sulphate transport gene (PDS). *Nature Genetics* 1997 **17** 411–422.
- 5 Bidart JM, Mian C, Lazar V, Russo D, Filetti S, Caillou B *et al.* Expression of Pendrin and Pendred Syndrome (PDS) gene in human thyroid tissues. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 2028–2033.
- 6 Royaux IE, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD *et al.* Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. *Endocrinology* 2000 **141** 839–845.
- 7 Kopp P. Pendred's syndrome: identification of the genetic defect a century after its recognition. *Thyroid* 1999 **9** 65–69.
- 8 Schmutzler C & Kohrle J. Implication of the molecular characterization of the sodium-iodide symporter (NIS). *Experimental and Clinical Endocrinology and Diabetes* 1998 **106** (Suppl 3) S1–S10.
- 9 Filetti S, Bidart J-M, Arturi F, Caillou B, Russo D & Schlumberger M. Sodium/iodide symporter: a key transport system in thyroid cancer cell metabolism. *European Journal of Endocrinology* 1999 **141** 443–457.
- 10 Arturi F, Russo D, Giuffrida D, Schlumberger M & Filetti S. Sodium-iodide symporter (NIS) gene expression in lymph-node metastases of papillary thyroid carcinomas. *European Journal of Endocrinology* 2000 **143** 623–627.
- 11 Fabien N, Fusco A, Santoro M, Barbier Y, Dubois P-M & Paulin C. Description of a human papillary thyroid carcinoma cell line. *Cancer* 1994 **73** 2206–2212.
- 12 Pang X-P, Hershmann JM, Chung M & Pekary AE. Characterization of tumor necrosis factor- α receptors in human and rat thyroid cells and regulation of the receptors by thyrotropin. *Endocrinology* 1989 **125** 1783–1788.
- 13 Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH & Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. *Journal of Clinical Investigation* 1993 **91** 179–184.
- 14 Weiss SJ, Philp NJ & Grollman EF. Iodide transport in a continuous line of cultured cells from rat thyroid. *Endocrinology* 1984 **114** 1090–1098.
- 15 Hedinger C, Williams ED & Sobin LH. The WHO histological classification of thyroid tumors: a commentary on the second edition. *Cancer* 1989 **63** 908–911.
- 16 Suarez HG, Daya-Grosjean L, Schlaifer D, Nardeux P, Renault G, Bos JL *et al.* Activated oncogenes in human skin tumors from a repair-deficient syndrome, *xeroderma pigmentosum*. *Cancer Research* 1989 **49** 1223–1228.
- 17 Arturi F, Russo D, Giuffrida D, Ippolito A, Perrotti N, Vigneri R *et al.* Early diagnosis by genetic analysis of differentiated thyroid cancer metastases in small lymph nodes. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1638–1641.
- 18 Arturi F, Russo D, Schlumberger M, Du Villard JA, Caillou B, Vigneri P *et al.* Iodide symporter gene expression in human thyroid tumors. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2493–2496.
- 19 Ponte P, Ng SY, Engel J, Gunning P & Kedes L. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. *Nucleic Acid Research* 1984 **12** 1687–1696.
- 20 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Annals of Biochemistry* 1976 **72** 248–254.
- 21 Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S *et al.* Expression of the Na⁺/I⁻ symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3228–3234.
- 22 Smanik PA, Liu Q, Furminger TL, Ryu K, Xing S, Mazzaferri EL *et al.* Cloning of the human sodium iodide symporter. *Biochemical and Biophysical Research Communications* 1996 **226** 339–345.
- 23 Caillou B, Troalen F, Baudin E, Talbot M, Filetti S, Schlumberger M *et al.* Na⁺/I⁻ symporter distribution in human thyroid tissues: an immunohistochemical study. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4102–4106.

- 24 Ryu KY, Senokozlieff ME, Smanik PA, Wong MG, Siperstein AE, Duh Q-Y *et al.* Development of reverse transcription-competitive polymerase chain reaction method to quantitate the expression levels of human sodium iodide symporter. *Thyroid* 1999 **9** 405–409.
- 25 Spitzweg C, Heufelder AE & Morris JC. Thyroid iodine transport. *Thyroid* 2000 **4** 321–330.
- 26 Russo D, Manole D, Arturi F, Suarez HG, Schlumberger M, Filetti S *et al.* Absence of sodium/iodide symporter gene mutations in differentiated human thyroid carcinomas. *Thyroid* 2001 **11** 37–39.
-

Received 19 January 2001

Accepted 18 April 2001